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THERAPY OF POSTIRRADIATION MARROW HYPOPLASIA WITH BLOOD COMPONENTS AND ANTIBIOTICS

J. E. Nutter, et al

Armed Forces Radiobiology Research Institute

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J. E. Nutter, R. G. Graw, Jr., and S. J. Baum

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Beagles exposed to 350 rads bilateral ^{60}Co gamma radiation without subsequent supportive treatment died with an average survival time of 14 days and with the expected signs of the hematopoietic syndrome: leukopenia, fever and bacteremia. Neither the administration of prophylactic antibiotics (sodium ampicillin and gentamicin sulfate) alone nor the transfusion of irradiated erythrocytes and platelets altered the mortality although the antibiotic regimen resulted in a small but significant mean prolongation of life to 18 days. When erythrocyte and platelet transfusions were administered in combination with the parenteral antibiotic regimen during the period of profound leukopenia, eight of nine treated animals survived. This combined regimen allowed autochthonous recovery of marrow function, observed 18 - 28 days postirradiation. Once myelogenous recovery had begun, the antibiotics were discontinued. Erythrocyte and platelet transfusions could usually be discontinued during the 4th week postirradiation. This simple therapeutic regimen resulted in a greater survival rate for beagles following uniformly lethal doses of irradiation than has been reported previously.

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THERAPY OF POSTIRRADIATION MARROW HYPOPLASIA WITH BLOOD COMPONENTS AND ANTIBIOTICS

J. E. NUTTER
R. G. CRAW, JR.*
S. J. BAUM

* National Institutes of Health
Bethesda, Maryland

S. J. BAUM
Chairman
Experimental Pathology Department

MYRON I. VARON
Captain MC USN
Director

ARMED FORCES RADIOBIOLOGY RESEARCH INSTITUTE
Defense Nuclear Agency
Bethesda, Maryland

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Exposure of mammals to ionizing radiation below 1000 rads will result primarily in injury to the blood-forming system. This induces a decrease in the formation of functional white cells, which are the first line of defense against bacterial infection. Consequently, the probability of infection and death is greatly increased.

On the other hand, if infections are prevented or controlled by the use of antibiotics, the bone marrow blood-forming system may recover from radiation injury and the animal may survive. Antibiotics alone are not as efficacious as antibiotics combined with red cell and platelet transfusions since radiation injury results in depression of these cell lines as well as white cells.

The objective of this study was to assess the efficacy of two antibiotics developed during the past decade, ampicillin and gentamicin, which had not yet been used in irradiated animals. Whenever indicated by signs of internal bleeding, this treatment was augmented by red blood cell and platelet transfusions and fluids.

Dogs were exposed to 350 rads of whole-body gamma radiation. Ampicillin and gentamicin treatments were begun at 6 and 12 days, respectively, in selected animals. All animals not receiving antibiotics died. However, only one of the nine dogs that received the combined treatment died, indicating that when these two antibiotics were administered as part of the previously described treatment regimen, it resulted in a significantly reduced death rate due to regeneration of the dogs' own bone marrow. By the 4th week postirradiation all surviving dogs showed evidence of adequate blood cell production, and antibiotic therapy was discontinued.

ABSTRACT

Beagles exposed to 350 rads bilateral ^{60}Co gamma radiation without subsequent supportive treatment died with an average survival time of 14 days and with the expected signs of the hematopoietic syndrome: leukopenia, fever and bacteremia. Neither the administration of prophylactic antibiotics (sodium ampicillin and gentamicin sulfate) alone nor the transfusion of irradiated erythrocytes and platelets altered the mortality although the antibiotic regimen resulted in a small but significant mean prolongation of life to 18 days. When erythrocyte and platelet transfusions were administered in combination with the parenteral antibiotic regimen during the period of profound leukopenia, eight of nine treated animals survived. This combined regimen allowed autochthonous recovery of marrow function, observed 18 - 28 days postirradiation. Once myelogenous recovery had begun, the antibiotics were discontinued. Erythrocyte and platelet transfusions could usually be discontinued during the 4th week postirradiation. This simple therapeutic regimen resulted in a greater survival rate for beagles following uniformly lethal doses of irradiation than has been reported previously.

I. INTRODUCTION

In most mammalian species, death caused by exposure to x- or γ-radiation doses below 1000 rads is primarily a result of damage to the hematopoietic system with a resultant pancytopenia. Leukopenia occurs early, and bacterial infections develop rapidly. The reduced peripheral granulocyte levels can be temporarily augmented with granulocyte transfusions, as shown by Epstein et al.,[4] and this may allow remaining stem cells to proliferate and repopulate the marrow if the animals have not expired from intervening infections.

Prophylactic administration of antibiotics to animals during the postirradiation period has proven to be beneficial but not uniformly successful as protection against infectious death;[2,6,7] consequently, it is important to select an antibiotic that is maximally effective. Ampicillin and gentamicin, which have been developed during the past decade, have been shown to be very effective in the treatment of septicemias caused by a variety of bacteria,[5] and this study was done, in part, to evaluate these antibiotics in the control of postirradiation infection.

Postirradiation anemia and thrombocytopenia must also be treated and controlled if animals are to survive long enough for bone marrow recovery. Therefore, red cell and platelet transfusions are required as a part of the total supportive regimen.

The objective of this study was to determine the effect of ampicillin and gentamicin, in combination with supportive care, in facilitating postirradiation survival of lethally irradiated beagles.

II. MATERIALS AND METHODS

Animals. Twenty-four 2 year-old purebred beagles of both sexes from the AFRRI colony were used. They had been immunized against distemper, infectious hepatitis, leptospirosis and rabies. Prior to initiation of the studies the animals were caged individually in temperature-controlled rooms, fed kibbled dog food, and provided water ad libitum. During the preexposure period they were observed for clinical illness and certified to be free of intestinal parasites. An experimental group of nine received the combined therapy of antibiotics and transfusions. Three groups of five comprised the controls: (a) antibiotics only, (b) whole blood and platelets, and (c) untreated.

Radiation exposure. The animals were exposed to 350 rads (midline tissue dose) of gamma radiation. During exposure the dogs were placed in Plexiglas boxes and were subjected to bilateral ^{60}Co gamma radiation at 40 rads/minute.

Observations. Experimental measurements were obtained at two preexposure intervals, daily for 30 days after exposure and then twice weekly for 30 additional days. The following measurements were made at each interval: body weight, rectal temperature, bacterial culture of blood, and a hemogram consisting of a hematocrit, total and differential leukocyte counts, and a determination of the number of platelets.

Therapy. The following treatments were used:

1. Antibiotics. Sodium ampicillin was administered as a subcutaneous dose of 0.5 g at 12-hour intervals beginning 6 days after irradiation. Gentamicin sulfate was given 12 days post-irradiation as 20 mg subcutaneously at 12-hour intervals. These schedules of ampicillin and gentamicin administration resulted in dose rates of

98-147 mg/kg per day and 3.8-5.3 mg/kg per day, respectively. Antibiotics were discontinued when the level of circulating granulocytes exceeded 1000/mm^3 for 48 hours.

2. Whole blood and platelets. These elements were transfused three times weekly during the period of decreased production. Donors and recipients were matched for canine erythrocyte antigen A, and the volumes were adjusted to 20 ml/kg. To increase the number of erythrocytes, whole blood was administered when the hematocrit fell below 30 percent. Platelet-rich plasma was prepared by centrifuging whole blood for 3 minutes at 1300 x g and was administered whenever platelet levels were less than 30,000/mm^3. When both the hematocrit and platelet values were below the limits, whole blood was administered. Whole blood and platelet preparations were irradiated with 2500 rads of 300 kVp x rays prior to administration to inactivate lymphocytes and to prevent graft versus host disease in the recipients.[1]

3. Fluid support. When a dog was observed to have both a progressive weight loss and a temperature elevation of 2° above base line, lactated Ringer's solution was administered. Forty milliliters per kilogram were usually administered subcutaneously twice daily but only once on those days when whole blood or platelet transfusions were also required.

III. RESULTS

Mortality. The data for the test and control groups are presented in Table 1 and are summarized in survival curves in Figure 1. All animals in the various control groups died 11 to 21 days postirradiation. Only one animal in the combined treatment group succumbed and it died on the 18th day postirradiation. In this population of beagles, 350 rads was a uniformly lethal dose of whole-body gamma radiation and the

combined therapeutic regimen was effective in markedly reducing the death rate to 12 percent. Neither component of the treatment, antibiotics or transfusions, when administered alone, altered the mortality but the antibiotics did induce a slight delay in the average day of death. Surviving, treated animals were observed in the animal

Table I. Postirradiation Mortality of Beagles Subjected to Various Treatment Regimens

Group	Number of survivors/ total number	Average day of death (range)
Untreated	0/5	14 (13-15)
Transfusions only	0/5	13 (11-15)
Antibiotics only	0/5	18 (13-21)
Combined treatment	8/9	18

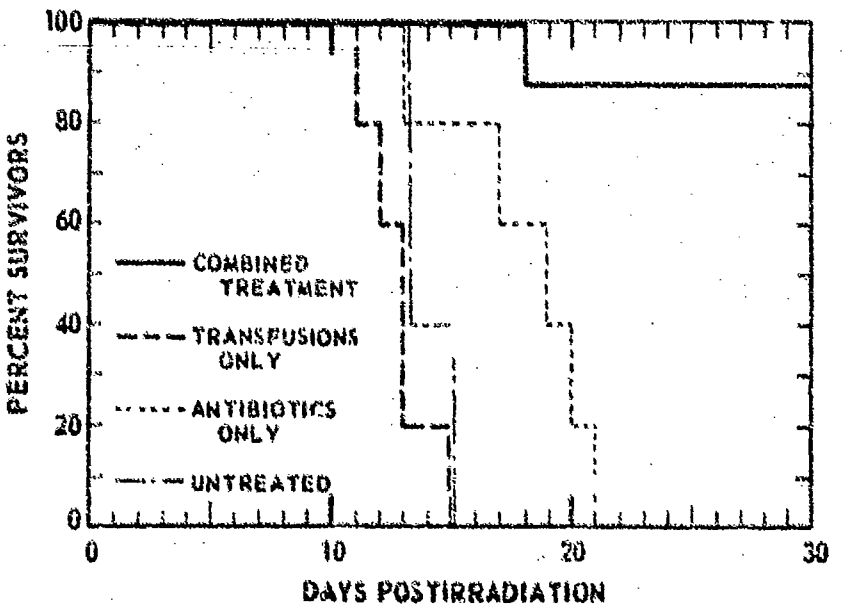

Figure 1. Postirradiation mortality and survival time of beagles subjected to various treatment regimens

facility at the AFRRI for at least 100 days after exposure and then were transferred to the animal farm for long-term observation. At the time of transfer, all were in good health and had no clinical signs of radiation sickness. They remained well for periods as long as 15 months postirradiation.

The cause of death, in the control animals and the one treated dog, was severe bilateral pneumonia. Antibiotic-resistant Klebsiella pneumoniae and Pseudomonas aeruginosa were the infective agents in animals that received antibiotics, whereas β-hemolytic streptococci and gram-negative enteric organisms were isolated from untreated dogs. Positive blood cultures were regularly obtained prior to death and correlated well with the tissue cultures obtained at necropsy.

Body weight. The general condition of the dogs correlated well with changes in body weight observed during the course of the study. All groups maintained nearly normal levels through the 7th day but subsequently lost weight progressively until death or until recovery of marrow function started. Beginning on the 30th day, the weight of survivors increased throughout the remainder of the 60-day observation period; however, only four of the eight dogs regained their preexposure weight. Vomiting and diarrhea were not observed in any group but the weight loss occurred in association with anorexia that developed after the 1st week.

Febrile response. The temperatures of the control and treated dogs are presented in Figure 2. The response of the combined treatment group was moderated throughout the course of the experiments when compared to values of the control animals. The highest mean value was 104.1 and was registered on the 19th and 20th days postirradiation. Six of these nine dogs registered temperatures of 105.0 but these

maxima were recorded at later times than was the case with any of the control animals. Mean values had returned to normal by the 25th day and remained at that level throughout the remainder of the observation period.

The criteria for administration of fluid, elevated temperature with a progressive weight loss, were met by three of the nine dogs receiving the combined therapy and fluid was administered during the period of 15 to 29 days.

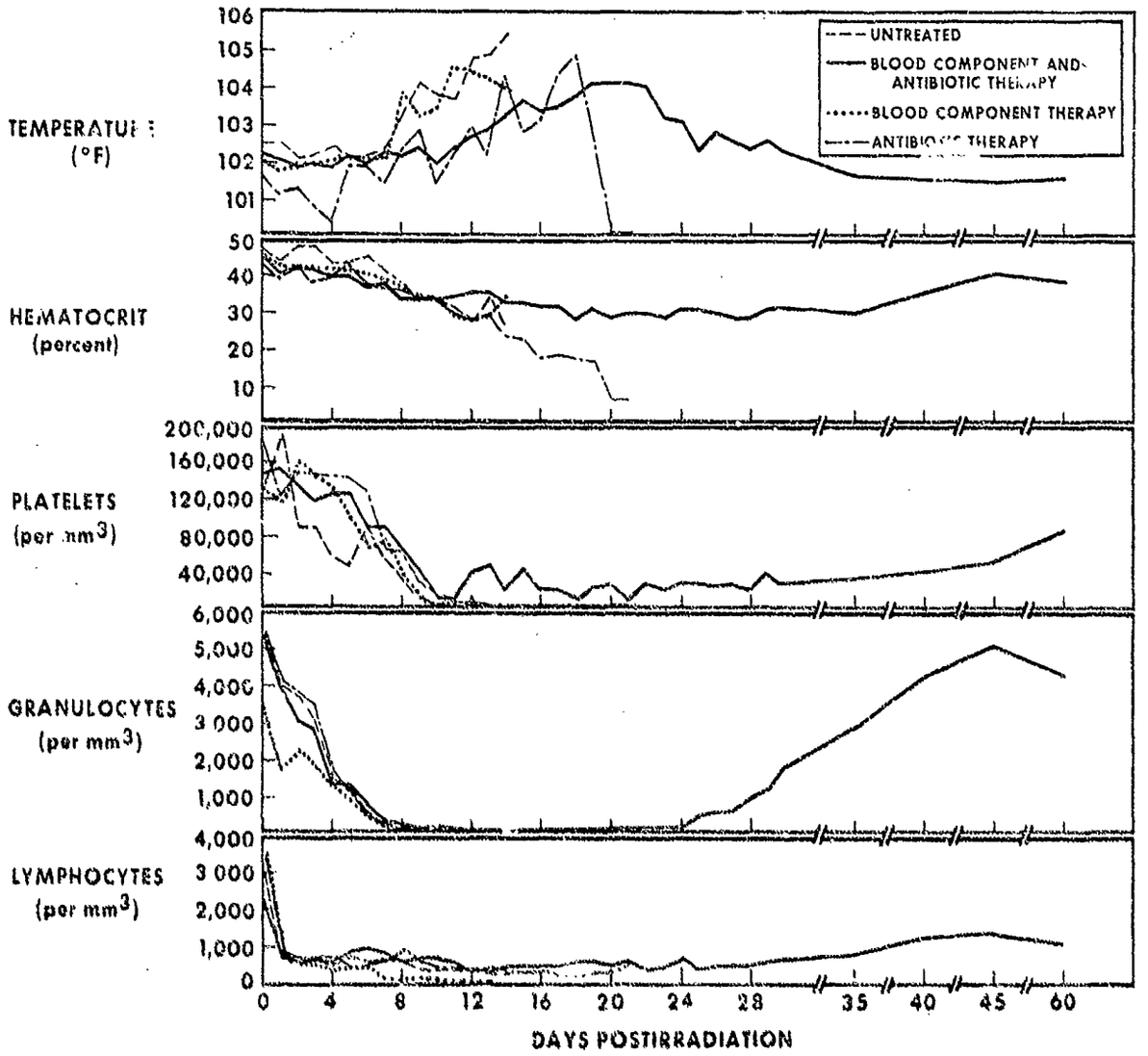

Figure 2. Postirradiation response of untreated and treated beagles

Hematocrit levels. Values for all dogs remained relatively stable for the first 4 - 6 days postirradiation but then showed a steady decline (Figure 2). The dogs that received no treatment and those that received only antibiotics had a progressive decline in values until death. Those dogs in the group that received only transfusions showed the same general response. Three of the five dogs in the transfusion control group met the criteria for transfusion prior to death and were given whole blood on the 12th day; however, these animals died before a second transfusion was required.

The hematocrit values for the dogs that received the combined treatment are less meaningful due to the administration of several whole blood and platelet transfusions. These transfusions were begun on the 10th or 11th day and were terminated at varying times from 16 - 37 days; the greatest frequency was during the 3rd week when every dog required three transfusions. This treatment maintained the hematocrits at approximately 70 percent of normal levels throughout the postirradiation period. Following cessation of the therapy, the values returned toward normal but only three of the eight survivors returned to preirradiation levels prior to the 60th day.

Platelets. The platelet levels declined throughout the early postirradiation period with the average levels dropping below 30,000/mm^3 on the 9th to 11th days for the control and treated groups (Figure 2). In the untreated and antibiotic control groups the platelets continued to fall until death; however, bleeding was not observed in these animals. Those control dogs which received a whole blood transfusion prior to death failed to demonstrate a significant increase in platelet levels. Platelet and whole blood transfusions maintained the platelet level at approximately 20,000/mm^3 in the combined treatment group during the period of marked thrombocytopenia. It

was observed that after 30 days, platelet transfusions were not required for platelet counts >10,000/mm^3 due to the improving condition of the animals. Following this criterion, only two dogs required platelet transfusions. During the 2nd month post-irradiation the platelet count rose steadily; however, only one of the eight surviving dogs reached preexposure levels. Bleeding was not observed to be a major problem with the treated dogs, but two of the survivors developed small hemorrhages at the injection sites.

Leukocytes. The number of lymphocytes was dramatically reduced 1 day post-irradiation to less than 40 percent of preexposure levels in all groups (Figure 2). Subsequently the numbers of lymphocytes remained rather stable until the animals in the three control groups died or until the 35th postexposure day when the mean value for the survivors of the combined treatment group showed evidence of recovery. Only one survivor regained preirradiation levels and at 60 days the group mean was only 50 percent of the preexposure value.

The granulocyte levels fell more gradually than did those of the lymphocytes following irradiation. In the three control groups the average number fell below 100/mm^3 on the 10th to 13th days. The mean values were similar for dogs in the combined treatment group; levels fell below 100/mm^3 on the 13th day and remained less than 100/mm^3 for 10 days when the mean values once again reached 100/mm^3. By day 28, the average values exceeded 1000/mm^3 which was evidence for recovery of hematopoiesis and therefore antibiotic therapy was discontinued. Prior to recovery these animals had no demonstrable circulating granulocytes for 3 to 15 consecutive days postirradiation. Autochthonous marrow recovery was first indicated by

circulating immature granulocytes observed at periods of from 19 to 28 days post-irradiation. Only four of the eight survivors had achieved preirradiation granulocyte levels before the end of the 60-day observation period.

IV. DISCUSSION

The therapeutic regimen used in this study was successful in reducing the mortality rate in beagles exposed to 350 rads whole-body ^{60}Co gamma radiation from 100 percent to 12 percent. It is felt that the major benefit was derived from the use of the antibiotics ampicillin and gentamicin. Gentamicin has been found to be particularly useful in those clinical situations where gram-negative bacterial infections are a problem. Since gram-negative organisms have been reported to be a particular problem in animals subjected to radiation or other immunosuppressive agents, it is felt that the use of antibiotics in an empirically applied regimen was the most significant factor in the recovery of the dogs.

The use of whole blood for erythrocyte and platelet transfusions to correct the deficiencies in erythrocytes and platelets and the use of lactated Ringer's solution as supportive fluid therapy are of secondary but critical importance. Dogs irradiated at this dose and treated with antibiotics alone had a prolonged survival time but all succumbed with extremely low hematocrit values or overwhelming infection. It is not felt that transfused granulocytes were a contributing factor in improving the survival of dogs that received the combined treatment. In preliminary studies in our laboratory, we have observed that 1×10^9 granulocytes transfused per day to similar dogs did not alter the survival rate. The number of granulocytes found in either the whole blood or platelet preparations was always far below 1×10^9.

These studies demonstrate the existence of stem cells surviving a uniformly lethal dose of whole-body gamma radiation and their capacity to repopulate the marrow. The antibiotics apparently did not suppress multiplication of the surviving stem cells because marrow recovery was observed at the same approximate time as in dogs surviving an LD_{50} dose without any treatment.[2,3] This therapeutic regimen can now permit studies of other functional capacities of dogs following usually lethal doses of irradiation and has obvious significance for the management of bone marrow recipients also subjected to immunosuppressive therapy.

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